FORM PTO-1390 (REV. 11-2000) U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: GJE-59

U S APPLICATION NO (If known, see 37 CFR 1 5): 09/786015

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/GB99/02729	9 March 2000	28 August 1998

TITLE OF INVENTION: High-Affinity Antibodies

APPLICANT(S) FOR DO/EO/US: Peter Harrison

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [X] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. [X] This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below.
4. [X] The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. [X] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [ ] is attached hereto (required only if not communicated by the International Bureau).
   b. [ ] has been communicated by the International Bureau.
   c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US).
6. [ ] An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. [ ] is attached hereto.
   b. [ ] has been previously submitted under 35 U.S.C. 154(d)(4).
7. [X] Amendments to the claims of the International Aplication under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. [ ] are attached hereto (required only if not communicated by the International Bureau).
   b. [X] have been communicated by the International Bureau.
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [ ] have not been made and will not be made.
8. [ ] An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371 (c)(3)).
9. [X] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)). unsigned
10. [ ] An English lanugage translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11 to 20 below concern document(s) or information included:

11. [ ] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [X] A FIRST preliminary amendment.
14. [ ] A SECOND or SUBSEQUENT preliminary amendment.
15. [ ] A substitute specification.
16. [ ] A change of power of attorney and/or address letter.
17. [ ] A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
18. [ ] A second copy of the published international application under 35 U.S.C. 154(d)(4).
19. [ ] A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
20. [ ] Other items or information:

U.S. APPLICATION NO (If known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
09/786015	PCT/GB99/02729	GJE-59

21. [X] The following fees are submitted:

	CALCULATIONS PTO USE ONLY
BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)):	
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO $1000.00	
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO $860.00	
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO $710.00	
International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) $690.00	
International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) $100.00	
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 860.00
Surcharge of **$130.00** for furnishing the oath or declaration later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	10 - 20 =	0	x $18.00	$ --
Independent claims	3 - 3 =	0	x $80.00	$ --
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ $270.00	$
TOTAL OF ABOVE CALCULATIONS =				$ 860.00
[ ] Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.			+	$
SUBTOTAL =				$
Processing fee of **$130.00** for furnishing the English translation later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				$
TOTAL NATIONAL FEE =				$ 860.00
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +				$
TOTAL FEES ENCLOSED =				$ 860.00
			Amount to be refunded:	$
			charged:	$

a. [ ] A check in the amount of $ _____ to cover the above fees is enclosed.

b. [X] Please charge my Deposit Account No. 19-0065 in the amount of $ 860.00 to cover the above fees. A duplicate copy of this sheet is enclosed.

c. [X] The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 19-0065. A duplicate copy of this sheet is enclosed.

d. [ ] Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137 (a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO Customer No.: 23557

Mr. David R. Saliwanchik
Saliwanchik, Lloyd & Saliwanchik
A Professional Association
2421 N.W. 41st Street, Suite A-1
Gainesville, FL 32606-6669

23557
PATENT TRADEMARK OFFICE

David Saliwanchik
SIGNATURE

David R. Saliwanchik
NAME

31,794
REGISTRATION NUMBER

February 28, 2001

Patent Application
Docket No. GJE-59

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s) : Peter Harrison

Docket No. : GJE-59

For : High-Affinity Antibodies

BOX PCT
Assistant Commissioner for Patents
Washington, D.C. 20231

PRELIMINARY AMENDMENT

Sir:

Please amend the above-identified patent application as follows:

In the Claims:

The following amendments are made with respect to the claims attached to the IPER. Therefore, please replace existing page 9 of the international application with the amended claim sheet (replacement page 9) of the annexes attached to the IPER, and make the following amendments to the claims including the replacement page 9.

Claim 2 (amended):

The [An] antibody, according to claim 1, wherein the amount of antibody bound in the second sample is > 60% of that bound in the first sample.

Claim 3 (amended):

The [An] antibody, according to claim 1 [or claim 2], wherein the pH in step (iii) is reduced to pH 2.5 - pH 2.0.

Claim 4 (amended):

The [An] antibody, according to [any preceding claim] claim 1, which is non-rodent.

Claim 5 (amended):

The [An] antibody, according to [any preceding claim] claim 1, which has affinity for a tumor-associated antigen

Claim 6 (amended):

The [An] antibody, according to claim 5, wherein the antigen is carcinoembryonic antigen.

Claim 7 (amended):

The [An] antibody, according to [any preceding claim] claim 1, which is a single-chain Fv, $F(ab')_2$, Fv or fab.

Claim 8 (amended):

The [An] antibody, according to claim 7, having a heavy chain variable region comprising the amino acid sequence defined in SEQ ID No. 2 and a light chain variable region comprising the amino acid sequence defined in SEQ ID No. 4, or a variant thereof having at least the same properties determined by the steps defined in claim 1.

Claim 9 (amended):

A polynucleotide molecule encoding [an antibody according to claim 8,] a high-affinity monoclonal antibody, wherein the affinity of said antibody is characterisable by:

(i) incubating first and second samples of the antibody in antigen-coated microtitre plate wells at a concentration chosen to be within the linear part of a standard curve at pH 7.2 for 1 hour at 37°C;

(ii) removing unbound antibody from both samples;

(iii) incubating the first sample with PBS at pH 7.2 for 1 hour at 37°C, and reducing the pH of the second sample to pH 3 or below and incubating for 1 hour at 37°C;

(iv) removing unbound antibody from both samples;

(v) incubating both samples with anti-antibody alkaline phosphatase-conjugate for 1 hour at 37°C;

(vi) removing unbound conjugate from both samples; and

(vii) adding PNPP substrate to the samples, measuring the absorbance of the samples at 405 nm, and determining the amount of antibody bound to antigen, wherein the amount bound in the second sample is > 50% of that of the first sample,

wherein said antibody has a heavy chain variable region comprising the amino acid sequence defined in SEQ ID No. 2 and a light chain variable region comprising the amino acid sequence defined in SEQ ID No. 4, or a variant thereof;

and wherein the polynucleotide comprises a nucleotide sequence defined in SEQ ID Nos. 1 and 3, or a variant thereof.

Claim 10 (amended):

A cloning vehicle comprising a polynucleotide molecule encoding a high-affinity monoclonal antibody, wherein the affinity of said antibody is characterisable by:

(i) incubating first and second samples of the antibody in antigen-coated microtitre plate wells at a concentration chosen to be within the linear part of a standard curve at pH 7.2 for 1 hour at 37°C;

(ii) removing unbound antibody from both samples;

(iii) incubating the first sample with PBS at pH 7.2 for 1 hour at 37°C, and reducing the pH of the second sample to pH 3 or below and incubating for 1 hour at 37°C;

(iv) removing unbound antibody from both samples;

(v) incubating both samples with anti-antibody alkaline phosphatase-conjugate for 1 hour at 37°C;

(vi) removing unbound conjugate from both samples; and

(vii) adding PNPP substrate to the samples, measuring the absorbance of the samples at 405 nm, and determining the amount of antibody bound to antigen, wherein the amount bound in the second sample is > 50% of that of the first sample;

wherein said antibody has a heavy chain variable region comprising the amino acid sequence defined in SEQ ID No. 2 and a light chain variable region comprising the amino acid sequence defined in SEQ ID No. 4, or a variant thereof;

and wherein the polynucleotide comprises a nucleotide sequence defined in SEQ ID Nos. 1 and 3, or a variant thereof.

The Commissioner is hereby authorized to charge any fees under 37 CFR 1.16 or 1.17 as required by this paper to Deposit Account 19-0065.

Respectfully submitted,

David Saliwanchik

David R. Saliwanchik
Patent Attorney
Registration No. 31,794
Phone No.: 352-375-8100
Address : 2421 N.W. 41st Street
Suite A-1
Gainesville, FL 32606

DRS/la

HIGH-AFFINITY ANTIBODIES

Field of the Invention

This invention relates to antibodies and their therapeutic use.

Background to the Invention

Antibodies have long been regarded as potentially powerful tools in the treatment of cancer and other diseases. However, although there have been some notable exceptions, this potential has not generally yet been realised.

This relative lack of success may be due, at least in part, to the use of monoclonal antibodies derived from rodents, which seldom have affinities higher than 10^{-9} M. Antibodies having this level of affinity are of limited therapeutic utility, as it has proved difficult to deliver enough antibody to the target to effect useful biological activity. Antibody binding to an antigen is reversible, and at the concentrations of antibody practical for *in vivo* use, dissociation will be favoured over association. In principle, it is possible to counter the dissociation of antigen by increasing the antibody concentration. However, this may lead to unacceptable clinical side-effects and would also increase the costs associated with the therapy.

Summary of the Invention

The present invention is based on the realisation that antibodies, or fragments thereof, can be produced which are "acid-resistant" and that this property is associated with high affinity binding of an antibody for its antigen.

According to the present invention, a high-affinity antibody has affinity characterised by:

(i) incubating first and second samples of the antibody in antigen-coated microtitre plate wells at a concentration chosen to be within the linear response part of a standard curve at pH 7.2 for 1 hour at 37°C;

(ii) removing unbound antibody from both samples;

(iii) incubating the first sample with PBS at pH 7.2 for 1 hour at 37°C, and reducing the pH of the second sample to pH 3 or below and incubating for 1 hour at 37°C;

(iv) removing unbound antibody from both samples;

(v) incubating both samples with anti-antibody alkaline-phosphatase conjugate for 1 hour at 37°C;

(vi) removing unbound conjugate from both samples; and

(vii) adding PNPP substrate to the samples, measuring absorbance of the samples at 405nm, and determining the amount of antibody bound to antigen, wherein the amount bound in the second sample is >50% of that of the first sample.

Preferably, the maximum pH in step (iii) is 2.5, more preferably 2.0.

Antibodies or antibody fragments with the "acid-resistant" properties are expected to favour association rather than dissociation and they therefore have longer localisation times at target sites, which results in a higher concentration of antibodies localised at the target sites.

In particular, this invention relates to the production of a high affinity single-chain Fv antibody fragment. This ScFv has particular advantages in that it allows better targeting to a site *in vivo*.

Description of the Drawing

Figure 1 illustrates the results achieved for acid-resistance of sheep and mouse monoclonal antibodies and single-chain Fvs with affinity to carcinoembryonic antigen at various pH values.

Description of the Invention

The acid-resistant monoclonal antibodies according to the present invention may be obtained using various techniques. For example, classical hybridoma technology can be applied, comprising the fusion of B-lymphocytes from immunised animals secreting high-affinity antibodies with an appropriate fusion partner. An alternative method is to purify the mRNA from selected lymphocytes and use the

technique of PCR to amplify the antibody genes required. Phage display technology and other techniques for the display of antibody fragments may also be used to obtain the antibody genes from naive or immunised libraries after appropriate selection procedures.

The antibody gene can be co-expressed with or otherwise chemically linked to toxins, radioisotopes or enzymes or any other desirable molecules to provide a fusion protein with strong binding characteristics. In a further alternative, the antibodies may be produced by transgenic animals as described in US-A-5770429.

The antibody may be a whole antibody, comprising heavy and light chains, and constant and variable regions. Alternatively, the antibody is an antibody fragment, e.g. $F(ab')_2$, Fab, Fv or single-chain Fv fragments, provided that at least part of the variable region is present which confers the property of "acid resistance". The antibody may also be an animal, chimeric or humanised antibody. A suitable method for producing humanised antibodies is disclosed in WO-A-92/15699.

In a preferred embodiment of the invention, the antibody is a single-chain Fv fragment. The single-chain Fv fragment comprises both heavy chain and light chain variable regions linked by a suitable peptide.

The antibodies of the present invention may be defined by their acid-resistant properties, which can be characterised by an acid-washed enzyme-linked immunosorbent assay (EIA), as described above. Typically the A_{405} value obtained by EIA will represent antibody binding of >50% for a sample at pH 3 or below, compared to the value for the sample at pH 7.2. Preferably, the A_{405} value of a sample at pH 2 will represent antibody binding of >60% more preferably 70% of that obtained at pH 7.2.

The animal that is subjected to immunisation is not a rodent, but is chosen to give higher affinity antibodies. Any large mammal may be used and suitable animals include rabbits, goats, cows and sheep.

An antibody of the invention may be used in therapy and may be formulated into any suitable composition with a physiologically-acceptable excipient, diluent or carrier.

The following Examples illustrate the invention.

Example 1.

Sheep were immunised with carcinoembryonic antigen (CEA) in complete Freund's adjuvant, then boosted three times with antigen in incomplete Freund's adjuvant. Animals were sacrificed after the final boost and lymph nodes removed.

The lymph node cells were then washed and fused with sheep heteromyeloma fusion partner SFP3.2. Fused cells were plated out at a total density of approximately 10^6 per ml in medium containing HAT (Life Technologies). These samples were then screened for hybridomas secreting high-affinity antibodies to the specified antigen using both a normal EIA and an acid-washed EIA.

Standard EIA screening assays were carried out as follows:

Maxisorb assay plates (NUNC) were coated with CEA (0.4 μg/ml in phosphate-buffered saline at pH 7.2), 100μl per well and left overnight at 4°C. The plates were then washed three times using phosphate buffered saline at pH 7.2 with 0.01% Tween 20 detergent. Any remaining reactive sites on the plates were blocked by the addition of 200μl per well of 0.2% fat-free milk protein in PBS at pH 7.2 at 37°C for ½ hour. The plates were then washed in PBS as described above and 45μl of the antibody samples were added to the wells of the plates. The samples were incubated for one hour at 37°C and then washed as described previously. Bound antibody was detected using alkaline phosphatase-conjugated donkey anti-sheep antibody (Sigma A5187 diluted 1/5000 in PBS at pH 7.2 with 1% BSA). The plates were then washed and 100μl per well of PNPP (Sigma N2770) solution was added. Absorbance was measured using a spectrophotometer at 405nm with phosphate buffered saline as a control.

Acid-wash EIA screening assays were carried out as follows:

Coating and binding of antibody samples was as described for the standard EIA above. However, after incubation with the antibody samples, the plates were washed and 200µl per well of HCl (10mM Stock solution) at pH 2 was added for one hour at 37°C. After three washes the antibody remaining bound to antigen was detected using alkaline phosphatase-conjugated donkey anti-sheep antibody and PNPP as described above. In order to ensure that a proper comparison was being made between antibodies at different concentrations, each sample was chosen to give an A_{405} value of approximately 1.0 in the normal EIA (i.e. in the linear response part of the EIA curve).

Three hybridomas (1D2, 6G11 and 6H9) secreted antibodies which gave a greater than 50% retention of binding in the acid washed EIA, in comparison to the binding in the non-acid washed EIA.

Example 2

A single-chain Fv fragment was produced from the hybridoma 6H9 above, as follows:

mRNA was purified from the cultured hybridoma cells using oligo-dT cellulose. Single-stranded DNA complementary to the mRNA (cDNA) was synthesized by reverse transcription. Universal primers, designed from the constant regions of sheep heavy and light chain antibody genes, were used in separate reverse transcription reactions to synthesise the cDNA for the antibody variable regions.

The cDNA was then amplified by the polymerase chain reaction to make double-stranded DNA using primers designed from the heavy and light chain variable framework sequences. Separate polymerase chain reactions were used to amplify the heavy and light chain regions. The products were then analysed by agarose gel electrophoresis and the DNA bands equivalent to light and heavy chain genes were cut from the gel and purified.

Equimolar amounts of variable heavy and light chain DNA were mixed together with an oligonucleotide linker DNA. The linker DNA coded for the amino acid sequence $(Gly_4Ser)_3$ with additional nucleotides complementary to the 3' end of the heavy chain variable region and the 5' end of the light chain variable region. The three DNA molecules were denatured, annealed and extended in the first stage (without primers) of a two-stage PCR reaction so that the fragments were joined, thereby assembling the single-chain Fv.

The single-chain Fv DNA was amplified in the second stage of the PCR using a pair of primers derived from the heavy and light chain variable region termini with the addition of the restriction enzyme recognition sites for *AlW44i* and *Not*I. The single-chain Fv gene product was analysed by agarose gel electrophoresis and purified. The single-chain Fv was then digested with the restriction enzymes *AlW44i* and *Not*I and cloned into an expression vector. The vector was then used to transform *E. coli* HB 2151, and protein expression was allowed to occur. The vector was designed so as to include a hexa-histidine tag at the COOH terminus of the SFv. The single-chain Fv was purified using nickel-chelate affinity chromatography and analysed by SDS-PAGE. The amino acid sequence for the heavy chain variable region and the light chain variable region is disclosed in SEQ ID Nos. 2 and 4, respectively. An acid-wash EIA was also carried out to determine the acid-resistant properties of the single-chain Fv.

Acid-wash EIA was carried out as follows:

Carcinoembryonic antigen (CEA)-coated microtitre plates were prepared as described previously. Single-chain Fv samples (6H9) were diluted to a range of concentrations between 1ng/ml and 100ng/ml in PBS at pH 7.2 containing 1% bovine serum albumin (BSA). 100μl samples were added to the microtitre plate wells and incubated for 1 hour at 37°C. The plates were then washed, 200μl per well of citrate added, and the plates incubated for 1 hour at 37°C.

In this case, the acid preparations were made using a stock solution of 100mM citrate diluted to pH values of 4.0, 3.5, 3.0, 2.5 and 2.0 in the reaction mixture. PBS at pH 7.2 was used as a reference control. The plates were then washed and 100μl per well of mouse anti-tetra-histidine antibody (Qiagen) (100ng/ml diluted in PBS at pH 7.2 with 1% BSA) added and incubated for 1 hour at 37°C. After plate washing the samples were incubated for 1 hour at 37°C with 100μl per well of goat anti-mouse alkaline phosphatase conjugate (Sigma A3688 diluted 1/1000 in PBS with 1% BSA at pH 7.2). The plates were then washed, treated with PNPP as described previously and the absorbance measured using a spectrophotometer at 405nm.

As a control for acid resistance, sFv samples were incubated with PBS at pH 7.2 to generate an EIA response curve for the SFv samples. In the linear region, a concentration of 10-20ng/ml of the SFv sample gave an absorbance (A_{405}) of 1.0-1.5 and was therefore used to determine the amount of antibody bound in the acid washed samples as a percentage of the amount bound in the reference sample.

The acid-resistant properties of the 6H9 whole antibody and the 6H9 single-chain Fv were compared with that for the mouse-derived anti-carcinoembryonic antigen whole antibody, A5B7 and the single-chain Fv MFE. The results are shown in Figure 1, with the antigen-binding of the mouse-derived antibodies being substantially reduced at pH 3.5 and less than 5% at pH 2.5. In contrast, the 6H9 antibodies retain >70% antigen at pH 3.5, >60% at pH 2.5 and >50% at pH 2.0.

CLAIMS

1. A high-affinity monoclonal antibody, wherein the affinity is characterisable by:

(i) incubating first and second samples of the antibody in antigen-coated microtitre plate wells at a concentration chosen to be within the linear part of a standard curve at pH 7.2 for 1 hour at 37°C;

(ii) removing unbound antibody from both samples;

(iii) incubating the first sample with PBS at pH 7.2 for 1 hour at 37°C, and reducing the pH of the second sample to pH 3 or below and incubating for 1 hour at 37°C;

(iv) removing unbound antibody from both samples;

(v) incubating both samples with anti-antibody alkaline phosphatase-conjugate for 1 hour at 37°C;

(vi) removing unbound conjugate from both samples; and

(vii) adding PNPP substrate to the samples, measuring the absorbance of the samples at 405nm, and determining the amount of antibody bound to antigen, wherein the amount bound in the second sample is >50% of that of the first sample.

2. An antibody according to claim 1, wherein the amount of antibody bound in the second sample is >60% of that bound in the first sample.

3. An antibody according to claim 1 or claim 2, wherein the pH in step (iii) is reduced to pH 2.5 - pH 2.0.

4. An antibody according to any preceding claim, which is non-rodent.

5. An antibody according to any preceding claim, which has affinity for a tumour-associated antigen.

6. An antibody according to claim 5, wherein the antigen is carcinoembryonic antigen.

7. An antibody according to any preceding claim, which is a single-chain Fv, $F(ab')_2$, Fv or fab.

8. An antibody according to claim 7, having a heavy chain variable region comprising the amino acid sequence defined in SEQ ID No. 2 and a light chain variable region

comprising the amino acid sequence defined in SEQ ID No. 4, or a variant thereof having at least the same properties determined by the steps defined in claim 1.

9. A polynucleotide molecule encoding an antibody according to claim 8, wherein the polynucleotide comprises a nucleotide sequence defined in SEQ ID Nos. 1 and 3, or a variant thereof.

10. A cloning vehicle comprising the polynucleotide molecule according to claim 9.

RE905827

USA

DECLARATION AND POWER OF ATTORNEY

As a below-named inventor, I hereby declare that my residence, post office address and citizenship are as stated below next to my name; I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of subject matter which is claimed and for which a patent is sought on an invention entitled
HIGH-AFFINITY ANTIBODIES

the specification of which ☐ is attached hereto or

☒ was filed on 20 AUG 1999 as United States Application Number or PCT International Application Number PCT/GB99/02729 and was amended on 31 AUG 2000 (if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above. I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56. I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for a patent or inventor's certificate, or PCT international application having a filing date before that of the application on which priority is claimed:

Prior Foreign Application Number(s)	Country	Foreign Filing Date	Priority Not Claimed	Certified Copy Attached? YES	NO
9818915.2	GB	28 AUG 1998	☐	☐	☐
			☐	☐	☐
			☐	☐	☐

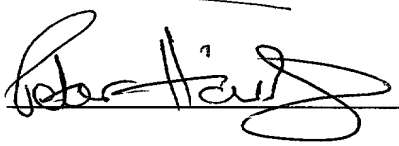
As a named inventor, I hereby appoint the following registered practitioner(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:
David R. Saliwanchik, Reg. 31,794; Jeff Lloyd, Reg. 35,589; Doran R. Pace, Reg. 38,261; Christine Q. McLeod, Reg. 36,213; Jay M. Sanders, Reg. 39,355; James S. Parker, Reg. 40,119 and Jean E. Kyle, Reg. 36,987; Frank C. Eisenschenk, Reg. 45,332; Seth M. Blum. Reg. 45,489

9

Direct all correspondence to:
Saliwanchik, Lloyd & Saliwanchik
2421 N.W. 41st Street, Suite A-1
Gainesville, FL 32606-6669
USA

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C 1001 and that such willful false statements may jeopardise the validity of the application or any patent issued thereon.

Full name of sole or First Inventor: Peter HARRISON

Inventor's signature: 

Residence address: Surrey, United Kingdom GBY

Post Office address: c/o KS Biomedix Ltd., 42-46 High Street, Esher, Surrey KT10 9QY, United Kingdom

Country of Citizenship: United Kingdom

Date of signature: 6.3.2001

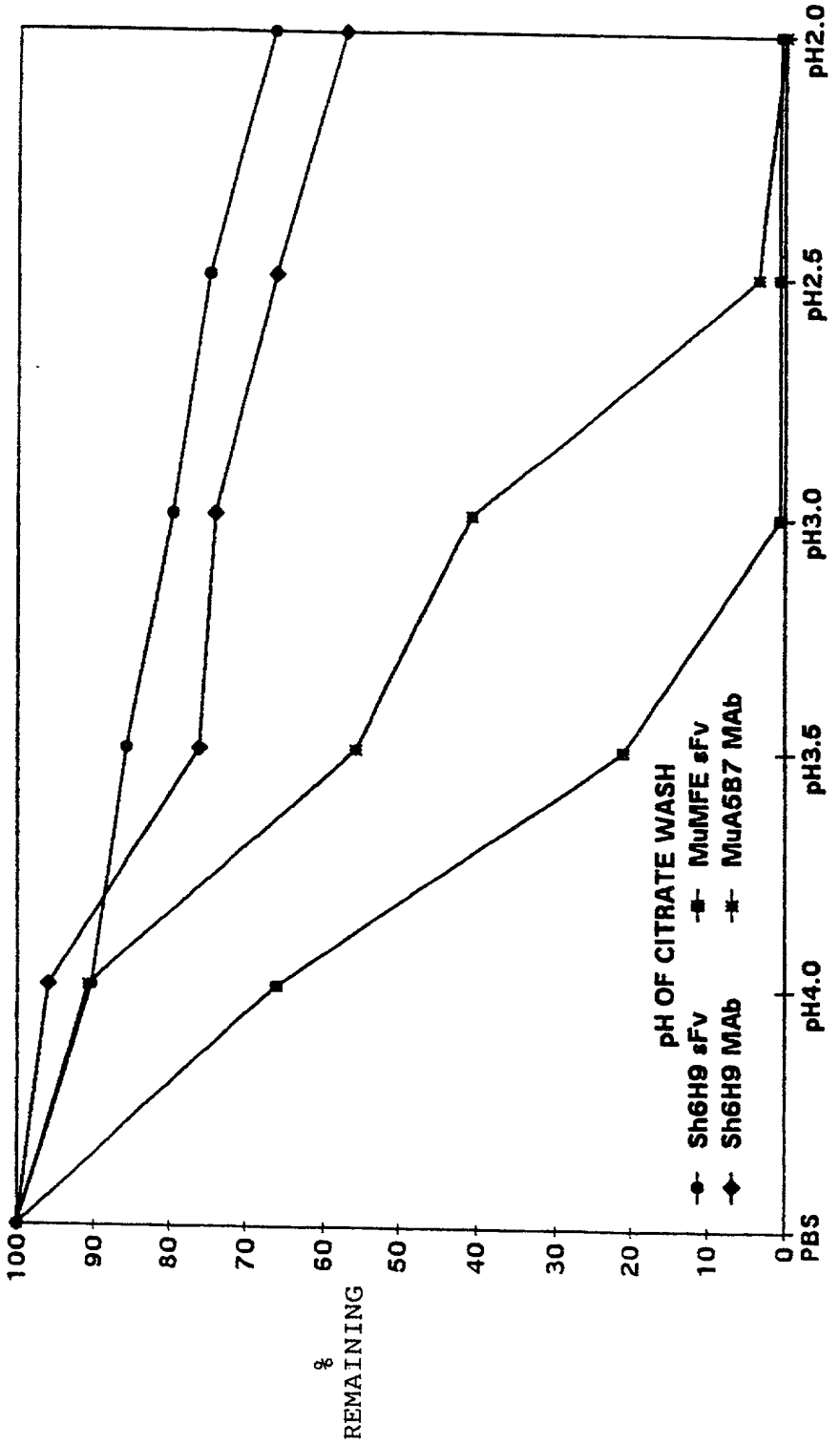

100
90
80
70
60
50
40
30
20
10
0
% REMAINING
PBS
pH4.0
pH3.5
pH3.0
pH2.5
pH2.0
pH OF CITRATE WASH
Sh6H9 sFv
Sh6H9 MAb
MuMFE sFv
MuA5B7 MAb

FIG. 1

SEQUENCE LISTING

<110> KS Biomedix Ltd

<120> ANTIBODIES

<130> rep05827wo

<140>
<141>

<160> 4

<170> PatentIn Ver. 2.1

<210> 1
<211> 363
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Antibody Fragment

<220>
<221> CDS
<222> (1)..(363)

<400> 1

```
cag gtg cag ctg cag gag tcg gga ccc agc ctg gtg aag ccc tca cag   48
Gln Val Gln Leu Gln Glu Ser Gly Pro Ser Leu Val Lys Pro Ser Gln
  1               5                  10                  15

acc ctc tcc ctc acc tgc acg gtc tct gga ttc tca tta acc aag tat   96
Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Thr Lys Tyr
             20                  25                  30

ggt gtt agt tgg gtc cgc cag gct cca gga aag gcg ctt gag tgg cta   144
Gly Val Ser Trp Val Arg Gln Ala Pro Gly Lys Ala Leu Glu Trp Leu
         35                  40                  45

ggt ggt gtg tcc agt ggt gca cta aca gcc tat aac aca gcc cta cag   192
Gly Gly Val Ser Ser Gly Ala Leu Thr Ala Tyr Asn Thr Ala Leu Gln
     50                  55                  60

tcc cga ctc agc gtc acc agg gac acc tcc aag agc caa ttc tcc ctg   240
Ser Arg Leu Ser Val Thr Arg Asp Thr Ser Lys Ser Gln Phe Ser Leu
 65                  70                  75                  80
```

```
tca ctg agc agc gtg act act gag gac acg gcc att tac tac tgt gcg   288
Ser Leu Ser Ser Val Thr Thr Glu Asp Thr Ala Ile Tyr Tyr Cys Ala
                85                  90                  95

aaa tct gtc aat ggt gac agt gtt cct tat ggt ttg gac tac tgg agc   336
Lys Ser Val Asn Gly Asp Ser Val Pro Tyr Gly Leu Asp Tyr Trp Ser
            100                 105                 110

cca gga ctc cta ctc acc gtc tcc tca                              363
Pro Gly Leu Leu Leu Thr Val Ser Ser
        115                 120
```

<210> 2
<211> 121
<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence:Antibody Fragment

<400> 2

```
Gln Val Gln Leu Gln Glu Ser Gly Pro Ser Leu Val Lys Pro Ser Gln
  1               5                  10                  15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Thr Lys Tyr
            20                  25                  30

Gly Val Ser Trp Val Arg Gln Ala Pro Gly Lys Ala Leu Glu Trp Leu
        35                  40                  45

Gly Gly Val Ser Ser Gly Ala Leu Thr Ala Tyr Asn Thr Ala Leu Gln
    50                  55                  60

Ser Arg Leu Ser Val Thr Arg Asp Thr Ser Lys Ser Gln Phe Ser Leu
65                  70                  75                  80

Ser Leu Ser Ser Val Thr Thr Glu Asp Thr Ala Ile Tyr Tyr Cys Ala
                85                  90                  95

Lys Ser Val Asn Gly Asp Ser Val Pro Tyr Gly Leu Asp Tyr Trp Ser
            100                 105                 110

Pro Gly Leu Leu Leu Thr Val Ser Ser
        115                 120
```

<210> 3
<211> 333
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Antibody Fragment

<220>
<221> CDS
<222> (1)..(333)

<400> 3

```
cag gat gtg ctg act cag ccg tcc tcc gtg tct ggg tcc ctg ggc cag   48
Gln Asp Val Leu Thr Gln Pro Ser Ser Val Ser Gly Ser Leu Gly Gln
  1               5                  10                  15

agg gtc tcc atc acc tgc tct gga agc agc agc aac att gga ggt aat   96
Arg Val Ser Ile Thr Cys Ser Gly Ser Ser Ser Asn Ile Gly Gly Asn
             20                  25                  30

gct tat gtg ggc tgg tac caa cag gtc cca gga tca gcc ccc aga ctc   144
Ala Tyr Val Gly Trp Tyr Gln Gln Val Pro Gly Ser Ala Pro Arg Leu
         35                  40                  45

ctc atc agt gct aca acc gat cga gcc tcg ggg atc ccc gac cga ttc   192
Leu Ile Ser Ala Thr Thr Asp Arg Ala Ser Gly Ile Pro Asp Arg Phe
     50                  55                  60

tcc ggc tcc agg tct ggg aac aca gcc acc ctg acc atc agc tcg ctc   240
Ser Gly Ser Arg Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Ser Leu
 65                  70                  75                  80

cag gct gag gac gag gcc gat tat tac tgt gca tcg tat caa agt act   288
Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ser Tyr Gln Ser Thr
                 85                  90                  95

tac agt ggt gtt ttc ggc agc ggg acc agg ctg acc gtc ctg ggt       333
Tyr Ser Gly Val Phe Gly Ser Gly Thr Arg Leu Thr Val Leu Gly
            100                 105                 110
```

<210> 4
<211> 111
<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence:Antibody

Fragment

<400> 4

```
Gln Asp Val Leu Thr Gln Pro Ser Ser Val Ser Gly Ser Leu Gly Gln
 1               5                  10                  15

Arg Val Ser Ile Thr Cys Ser Gly Ser Ser Ser Asn Ile Gly Gly Asn
            20                  25                  30

Ala Tyr Val Gly Trp Tyr Gln Gln Val Pro Gly Ser Ala Pro Arg Leu
        35                  40                  45

Leu Ile Ser Ala Thr Thr Asp Arg Ala Ser Gly Ile Pro Asp Arg Phe
    50                  55                  60

Ser Gly Ser Arg Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Ser Leu
65                  70                  75                  80

Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ser Tyr Gln Ser Thr
                85                  90                  95

Tyr Ser Gly Val Phe Gly Ser Gly Thr Arg Leu Thr Val Leu Gly
            100                 105                 110
```